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Introduction: The Molecular Genetic Analysis of Mouse Development

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We are witnessing an extremely exciting period in the analysis of mammalian development. A number of factors are contributing to this burst of activity. Foremost is the infusion provided to the field from the employment of new technologies, including those based on recombinant DNA technologies, PCR technologies and "gene targeting." Second, there is an enormous influx of information coming from the molecular genetic and embryological analyses of other organisms, notably yeast, *C. elegans*, *Drosophila, Xenopus*, and chick. A decade ago no one would have predicted the extent to which the molecular circuitries that are used to mediate cell-cell interactions, intracellular signal transduction, specification of positional information along the embryonic axes, or cellular differentiation are conserved in all animal species. So profound is this conservation that the discovery of a molecular circuit in one organism immediately initiates a search for the same pathway in all of the others.

However, despite the undeniably important contributions emanating from the analyses of other organisms and the power of comparative analysis, mechanisms of mammalian development must, for seveal reasons, be studied in mammals, principally in the mouse. First, many aspects of mammalian development may be unique to mammals.

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Early mammalian development is distinguished by the establishment of an intimate connection between the conceptus and the uterus of the mother. This point of exchange provides a virtually infinite source of energy to the developing embryo which allows enormous growth during development. As a consequence, we may anticipate that patterning of the mammalian embryo may be more tightly coupled with growth than is observed in other organisms. Second, the initial embryonic cell cleavages generate an apparently equivalent set of cells that have no obvious axes and are depleted of maternal messengers. Thus, in contrast with the development of many other organisms, maternal embryonic factors are not likely to be important contributors to the patterning of the mammalian embryo. Third, although many molecular circuits are likely to be conserved between ourselves and other species like *Drosophila* , the way in which these circuits are used to guide patterning of systems such as the brain may not be conserved. After all, six hundred million years have elapsed since the divergence of vertebrates and invertebrates. This seems ample time for evolution to have found new uses for old parts. Fourth, because we are mammals, it is not unexpected that we should find mammalian development particularly interesting. This stems not only from an innate curiosity about ourselves and how we are formed, but also from the practical consideration that application of embryological insights to human medicine will require detailed knowledge of the developmental process within a mammalian species.

The difficulties associated with working in mammalian development are well known and numerous: lengthy generation times, complexity of the organism, relative inaccessibility of the post-implantation embryo, husbandry costs, and so on. However, such a list of handicaps merely provides a set of challenges for the investigator. Considering our own generation time, complexity, partner preferences, and rearing costs, the mouse provides a fabulous alternative. This was recognized well over a century ago and accounts for the existence of the numerous well-established resources such as inbred strains of mice, an extensive mutant collection, a robust linkage map, and a detailed

anatomical description of development. It is within the framework of this rich heritage that molecular geneticists and molecular biologists are applying their new tools.

The molecular analysis of mammalian development is in its infancy. Nevertheless, the nature of the information being generated through this approach and the directions the field is taking are emerging. We can now detect with precision the expression patterns of genes mediating development and thereby define their potential regions and times of influence. Molecular markers can also be used to define the identity and behavior of populations of cells during development with a precision not attainable by other techniques. Mutational analysis then allows systematic dissection of the developmental process and determination of gene function.

This collection of excellent papers provides us with a snapshot of the field of mouse development as it is practiced today. Obviously the entire field cannot be represented in seven papers. Nevertheless, a remarkably wide spectrum of topics is covered. The first paper, by Janet Rossant, describes very early mouse development. Most of this period is directed at elaborating the extraembryonic lineages critical for the continued survival of the conceptus in the uterine environment. Thus, it is not surprising that most mutations affecting the formation and continued development of the conceptus result from defects in the extraembryonic tissues. Janet Rossant provides an elegant criterion for distinguishing whether a mutation that affects embryonic development is intrinsic to the embryo or occurs as a result of a defect in extraembryonic tissue. If chimeras produced by aggregation of mutant embryos with tetraploid wild-type embryos phenotypically rescue the mutant embryo, then the defect is in extraembryonic tissues. This follows because tetraploid cells are excluded from the embryo proper but can contribute to the formation of trophectoderm and extraembryonic endoderm derived tissues. Tetraploid embryos can be easily generated by electrofusion of two-cell stage embryos. Using this technique, Rossant and her colleagues demonstrated that disruption of *Mash-2* leads to defects in extraembryonic tissues only. Dr. Rossant also provides a

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lucid review of the tissue-specific transcription factors and of the cell signaling pathways that contribute to the formation of the extraembryonic cell lineages. Because the elaboration of the extraembryonic tissues involves the formation of relatively few cell types, this system is particularly tractable to genetic and molecular analysis. However, since the emergence of this system is a relatively late innovation in evolution, we may anticipate the juxtapositioning of unexpected molecular partners. Evolution can be capricious in her choice of molecular hardware to mediate innovation.

Next, Frank Conlon and Rosa Beddington provide an intriguing and insightful comparison of gastrulation in *Xenopus* and mouse. This is a particularly fascinating period of development since it sets the stage for the emergence of a common vertebrate body plan. Lewis Wolpert has said, "It is not birth, marriage or death, but gastrulation which is the truly important event in your life." Interestingly, *Xenopus* and mouse enter gastrulation from very different points in development but emerge with a common body plan. This suggests that initiation of gastrulation in the two organisms may be different, perhaps utilizing different molecular players, but that eventually the process must funnel into a common program. Perhaps the emergence of Spemann's organizer in *Xenopus* and the node in the mouse point to a common mesoderm patterning mechanism. The interplay between developmental studies in these two organisms continues to generate interesting discourse, and a number of developmental biologists have made very successful careers by working in both organisms. Not only are the pregastrula states quite different in the two organisms, but the experimental paradigms used for analysis are also very different. In *Xenopus,* tissue manipulations, either in the intact embryo or in culture, coupled with the use of molecular markers provide the critical investigative tools for analyzing early inductive events. In the mouse, molecular genetic analysis is emerging as a primary tool of the trade. However, mouse embryo manipulation *in utero* and in culture is increasing. The use of different experimental paradigms in *Xenopus* and mouse compounds the difficulties of comparative analysis. Nevertheless, the two

systems continue to play synergistic roles. A recent example is the genetic demonstration by Bradley and his colleagues that activin does not appear to perform a critical role in mesoderm induction in the mouse, which brings into question such a role in *Xenopus*. As a consequence, the spotlight is shifting to other members of the *TGF-β* super family in both organisms.

In the third paper, Brigid Hogan reviews the roles of members of the *TGF-β* super family in mouse development. What emerges from her excellent review is the enormous complexity of this field, due not only to the expanding membership of this gene family, but also to the growing number of known points at which the activity of the individual TGF-β gene products can be regulated. These include the processing, sequestering and presentation of the ligand gene product to a multitude of receptor combinations. At each point several gene products can be involved, including proteases, protease inhibitors, ligand binding proteins, and extracellular matrix proteins. Finally, following the activation of the receptor complex, a multitude of cellular responses can ensue. Faced with interactions as complex as these, it is tempting to throw up one's hands in despair of ever making meaningful progress in unraveling all the circuits involved in an organism itself as complex as a mouse. However, without researchers willing to jump in and tear the system apart, meaningful advances are guaranteed not to occur. The advent of more sophisticated means for manipulating the mouse genome, such as tissue-specific gene disruptions and the placement of genes under the control of inducible switches, should facilitate the unraveling of these complex developmental problems. Brigid Hogan makes the interesting suggestion that variations in the *TGF-β* activity modifying factors could account for the observed variation of the penetrance and/or expressivity of a mutant phenotype in different genetic backgrounds. She illustrates the range of developmental functions mediated by *TGF-β* family members by discussing the phenotypes of mice with mutations in *TGF-β1, BMP5* and Nodal. Hogan concludes her article by articulating our

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ignorance about the ways in which the *TGF-β* system interacts with other cell-cell signaling systems such as the *Wnt* family members.

In the fourth paper, McMahon and his colleagues provide us with a clear overview of the role of the *Wnt* family members in mouse development. These genes encode secreted glycoproteins and are implicated in mediating critical functions during gastrulation, CNS patterning, organogenesis and limb development. Thus, these signaling molecules, like those of the *TGF-β* family members, are performing a wide array of functions during mammalian development. McMahon focuses on genetically defining the functions of several *Wnt* family members. Curiously, *Wnt-3a* mutant embryos show truncation of the body axis caudal to the forelimbs, with somite-derived tissues being particularly affected. McMahon suggests that the absence of defects rostral to the forelimbs indicate that the more rostral mesoderm-derived tissues must be patterned around the node prior to normal *Wnt-3a* expression. *Wnt-1* has been shown to be critical for patterning the CNS. In the absence of *Wnt-1* gene product, caudal midbrain and the rostral portion of the hindbrain are not formed. Consistent with its role as an oncogene, these observations implicate *Wnt-1* in controlling proliferation of precursor cells responsible for forming this portion of midbrain and rostral hindbrain. Although many *Wnt* family genes show intriguing overlapping expression patterns in the CNS, the role of these genes in patterning the CNS has not yet been determined. Most recently, McMahon and his colleagues have demonstrated a role of *Wnt-7a* in the dorsoventral patterning of the limb. *Wnt-7a* mutant mice show a dramatic duplication of ventral limb structures, such as foot pads, sesamoid bones and tendons, on the dorsal half of the mutant foot. Here is a clear example of *Wnt* genes regulating cell fate.

The role of *Hox* genes in patterning the branchial region of the head is expertly summarized by Mark, Rijli and Chambon. Their review concentrates on the functions of *hoxa-1, hoxa-2* and *hoxa-3* in mouse development. Loss of function mutations in *hoxa-1* suggest a role for this gene in establishing or maintaining the segmental pattern in the

hindbrain (i.e., the number of rhombomeres). Such a role is in stark contrast to the situation in *Drosophila* where *HomC* genes, homologues of the vertebrate *Hox* genes, are not involved in the segmentation process. Thus in *Drosophila,* mutations in *HomC* genes can change the identity of parasegments, but do not change the number of parasegments. *Hoxa-2* mutations, however, do appear to change cell fates. In mice mutant for *hoxa-2*, arch 2 mesectoderm-derived structures appear to be transformed towards arch 1 type structures. This results in the production of curious mirror image duplications of a subset of arch 1 type structures. The authors suggest that these transformations reveal the existence of an arch 1 ground state patterning program common to at least arch 1 and arch 2. In addition, their mutant mice also showed a new ectopic cartilage that resembled a reptilian ptergoquadrate element revealing an atavistic aspect of the *Hox* developmental program. Chambon and his colleagues provide a provocative synthesis of these observations by suggesting that the function of *Hox* genes can be placed in an evolutionary context to understand the history of vertebrates. For example, the presence of a first arch ground state common to both arch 1 and arch 2 is consistent with the thesis that these two arches are serially derived, homologous structures that share a common morphogenetic program. This suggestion, in turn, is consistent with the view that arch 1 and arch 2 could be true homologues of the original gill-bearing arch present in agnathan ancestors of gnathostomes.

The sixth paper, by St-Onge, Tuello and Gruss, reviews the role of *Pax* genes in mouse development. These genes encode transcription factors whose common DNA binding motif is called the paired box. In addition, many members of this family also contain a second DNA binding motif belonging to the homeodomain class. To date, nine *Pax* genes have been identified (*Pax-1* to *Pax-9*). Most have intricate patterns of expression in the spinal column and the brain. Three pre-existing mouse mutations, *undulated, splotch* and *small eye*, and two human diseases, *Waardenburg syndrome* and *aniridea*, have been shown to result from mutations in *Pax* genes, emphasizing their

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important roles in neurogenesis, myogenesis and organogenesis. Primarily from elegant work emanating from Peter Gruss' laboratory, the ways in which these gene products function as transcription factors is also being elucidated. Considering that many members have two DNA binding domains, each capable of independent as well as synergistic and antagonistic interactions, the repertoire of regulatory functions mediated by these genes is likely to be very complex. The expression patterns of *Pax* genes in the spinal cord are particularly fascinating. Some are expressed early in embryogenesis (*Pax-3, Pax-6* and *Pax-7*) whereas others are expressed later (*Pax-2, Pax-5* and *Pax-8*). Their expression patterns also vary along the dorsoventral axis suggesting that they may play important roles in dorsoventral patterning of the spinal cord. Consistent with this hypothesis, Peter Gruss' laboratory has shown that *Pax* gene expression is responsive to signals emanating from the floor plate and the notochord, important sources of inducing signals that mediate dorsoventral patterning of the spinal cord. The above is but one example of the role of these molecules in mammalian development. In addition to defining the individual roles performed by these transcription factors, it will be important to determine how these factors interact with other gene families, such as the *Hox* genes, and whether such interactions can be grouped into patterns associated with functional themes.

The last paper is by Elizabeth Robertson. This fascinating report provides a description of how two growth factors, IGFI and IGFII and their receptor IGF1R interact to control the growth of the embryo and extraembryonic tissues. In addition, a second receptor, IGF2R/MPR, appears to be involved in modulation of the level of IGFII circulating in the embryo by internalization and degradation. To complicate matters, both IGFII and IGF2R/MPR are imprinted, but with opposite polarities. Robertson and her colleagues disrupted IGFI, IGFII and IGF1R. In addition a deletion mutant encompassing IGF2R gene already existed. As a consequence, they were able not only to determine the individual roles of these genes in embryonic growth, but also to study the

interactions between these genes by making the appropriate double mutants. For example, they demonstrate *in vivo* that both IGFI and IGFII function through IGF1R, but that in addition, IGFII functions through an unidentified receptor. They could also conclude that IGF2R/MPR mutations are lethal as a consequence of excess circulating IGFII. These elegant studies made the unexpected prediction that IGF2R/MPR mutant mice should be rescued by a IGFII mutation. By making the double mutant, Robertson and her colleagues showed this prediction to be true .

I hope I have piqued your interest in reading this collection of excellent papers. You will find their contents to be not only of scientific interest but also reflective of the current state of the field. We are at the early stages of information gathering. Investigators are working within their particular fields of expertise, and the points of intersection are not yet apparent. But that will come. This is a necessary stage because detailed descriptions of the individual processes are a prerequisite for deeper insights into their likely interactions.

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